

ART. VII.—*On Bacterium Solanacearum Smith as the Causal Agent of the Brown Rot Disease of Potatoes in Victoria.*

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A disease of potatoes known as Brown Rot or Sore Eye has been known to occur in Victoria for many years.

McAlpine (2) recognized that it was bacterial in nature, and concluded from a study of the symptoms that it was identical with a disease of potatoes in the United States caused by *Bacterium solanacearum* E.F.S. This conclusion was never definitely established however, as, although McAlpine sent typically diseased tubers to Dr. E. F. Smith in the United States of America, they were so badly rotted on arrival that an isolation of the causal agent was impossible.

The same disease has been recorded in other Australian States. Tryon (5) described the disease in Queensland in 1894, and, as will be noted later, gave a name to the bacterial organism he isolated. Darnell-Smith (1), while investigating a bacterial disease of tobacco in New South Wales, made some isolations from Brown Rot infected potato plants, but seemed uncertain as to whether the organism isolated was really *B. solanacearum*.

This paper offers proof of the pathogenicity of a bacterial organism in connexion with Brown Rot of potatoes, and the organism is identified.

Symptoms of the Disease.

One of the earliest symptoms on the leaves is a bronzing, which is followed by browning and shrivelling. Later on, leaves and petioles show a wilted appearance. On cutting across the stem, a whitish bacterial ooze often becomes manifest. The bacteria are seen to be located in the vascular bundles. Young tubers show small amounts of soil adhering to them in the region of the eyes. In older tubers, especially in damp soil, the eyes show a whitish matter which exudes through the adhering soil. This appearance is responsible for the popular name Sore Eye or Spewey Eye. On cutting across such an infected tuber, a greyish white bacterial ooze is to be seen coming from the bundles. Infected tubers on storage undergo a soft rot and give off an unpleasant odour.

Pathological Histology.

Transverse sections cut through the stems, roots and stolons, show the bacteria to be restricted to the vascular wood elements. In older diseased parts, break-down of the wood elements may occur, and other tissues may be destroyed leading to the formation of cavities containing bacteria.

The wilting of the plant appears to be in part caused by the clogging of the vessels with bacteria. Infection spreads from the stem out through the stolons, and the whole vascular ring of the tuber shows the presence of organisms. Breakdown of tissue occurs in the vicinity of the eyes, the buds being in many cases destroyed. The bacteria then ooze out on the surface of the tuber in the region of the eyes.

Isolation of the Organism.

The causal organism was consistently isolated from the vascular system of young infected tubers when they were opened aseptically. The organism was also obtained from the bacterial ooze on cutting across the stems of wilted plants. Pathogenicity was established for potato plants and also for certain other plants as detailed later.

Description of the Causal Organism.

The organism is a gram negative cocco-bacillus (1 to 1.4μ in length x $.7\mu$ in diameter) which usually occurs in pairs. It is motile by a single polar flagellum.

Colonies appear somewhat slowly on beef agar plates (64 hours). They are circular, smooth, wet-shining and white in colour. On beef agar slopes (pH 7.2) the growth is good. The culture shows a white moist growth of a semi-fluid consistency. After some days, a clearing zone develops outside the edge of the culture, and outside this again, extending over the rest of the agar surface, appears a pale white substance. This substance was found to be soluble in dilute acetic acid.

The organism is aerobic, since, in agar slabs good growth occurs at the surface, and only a little in the depth. Nutrient broth showed uniform clouding in 24 to 48 hours, but no pellicle was formed. Growth on potato slopes is moist and spreading, and is at first of a whitish yellow colour. A very faint brown pigment develops after two or three days. Litmus milk shows blueing, but no clot occurs. There is little or no growth in Uchinsky's solution. In the case of two isolations however, the organism, which was definitely pathogenic, grew well in this medium. In Cohn's solution the organism fails to grow. Colonies on gelatine plates are small and white; no liquefaction is observed in gelatine for three or four weeks, but, after this time, slight liquefaction has been found to occur. Neither acid nor gas is

formed in lactose, galactose or saccharose. Some acid is formed in glucose after several days' growth, but this is not constant for different isolates of the same organism. Nitrates are reduced, but no indol or hydrogen sulphide are produced. The life of the organism is short, being two to five weeks, and it very rapidly loses its virulence. The thermal death point is 51°C.

Inoculation Experiments.

The organism was inoculated by needle pricks into the stems of healthy potato plants, and the typical disease was reproduced after periods ranging from a fortnight up to two months.

Host Range.

Artificial inoculations by needle pricks produced a wilt, and vascular bacteriosis of garden nasturtium (*Tropaeolum majus*) tomato, castor oil plant, and black nightshade.

Infection of garden nasturtium leaves was also easily obtained by spraying with a suspension of the organism. The incubation period of the disease for garden nasturtium under conditions favorable for infection, varied between one and three weeks; for tomato, seven to twelve days, and for castor oil plants, ten to nineteen days. In tomato, the infected petioles showed a characteristic downward bending of an epinastic type, while adventitious roots appeared on the stem some time before any wilting was apparent. The physiology of these phenomena is being investigated and the results will be published later. Petioles also showed brownish streaks in the earlier stages of the disease. Repeated inoculations of tobacco, and sunflower plants, failed to produce any symptoms of disease.

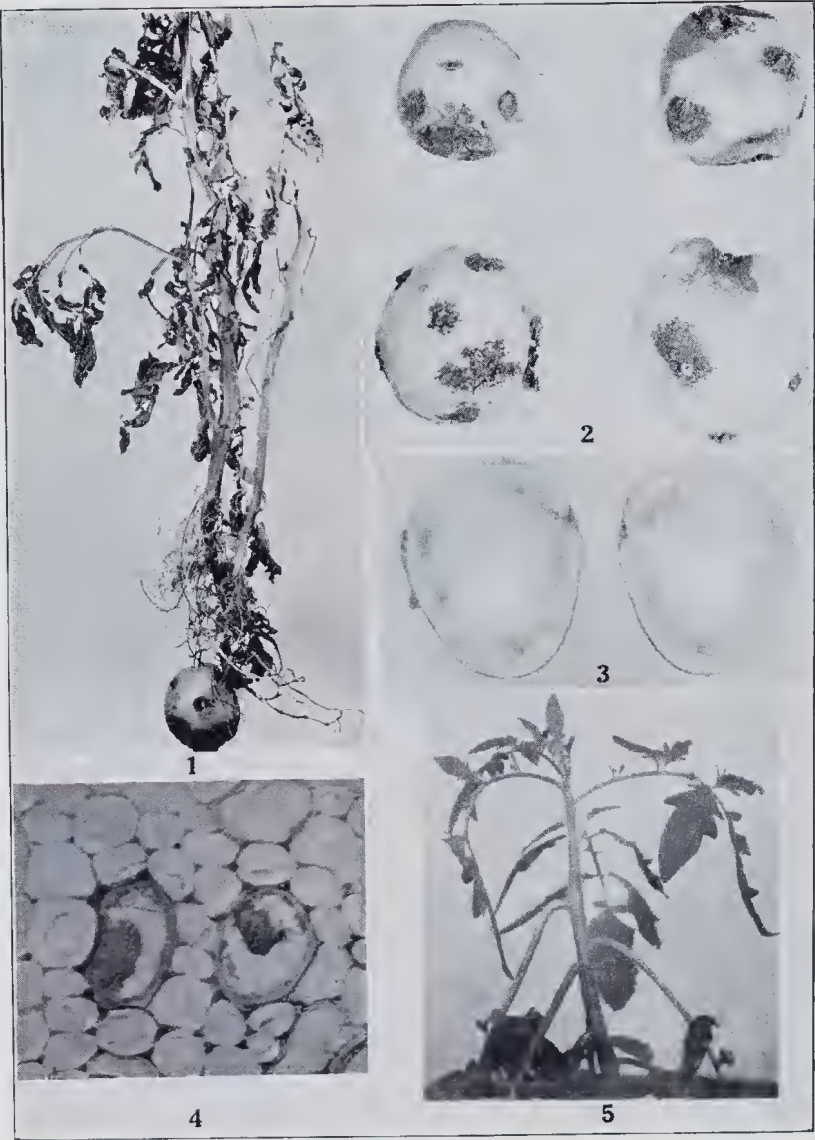
Discussion.

In its cultural and physiological characters the organism causing Brown Rot or Sore Eye of the potato differs from *Bacterium solanacearum* E. F. Smith (*Phytophthora solanacearum* (E.F.S.) Bergey et al.) in that, (a) no water soluble brown pigment commonly forms in colonies on beef agar plates, (b) a slight amount of acid is formed in glucose, (c) certain isolates in which the organism was pathogenic showed good growth in Uschinsky's solution. To these differences must be added the fact that the organism responsible for Brown Rot of potato in Victoria will not infect tobacco. Welles and Roldan (7) have described an isolate from Tango (*Chrysanthemum coronarium*), in the Philippines which does not produce brown pigment on beef agar, although in its other characters it agrees with *B. solanacearum*. They consider this difference sufficient to call their organism a strain. Nolla (3) working on a bacterial wilt of Solanaceous plants in Porto Rico found that the organism isolated, while agreeing in its cultural and physiological characters with *B. solanacearum*, would not infect tobacco. This

inclined him to the view that he was dealing with a distinct strain of the type organism.

In view of the fact that the organism isolated in Victoria shows both these differences as well as a difference in the fermentative properties, it is felt that it should be regarded as a distinct strain of *B. solanacearum* E.F.S. Darnell-Smith's description (1) of an organism isolated on one occasion from a diseased potato plant agrees essentially with the author's description. It clotted litmus milk, and, after a period of subculture, produced acid in glucose and lactose, and bleached litmus milk. Uncertain as to the importance of the differences observed, Darnell-Smith made no definite pronouncement on the identity of his organism. Furthermore, no inoculation experiments were described. The organism he isolated from diseased tobacco plants showing symptoms of Brown Rot shows rather striking differences in cultural and physiological characters from the type culture, and suggests a different organism, or at least a widely divergent strain. The fact that the organisms he describes from diseased tobacco plants, and from diseased potato plants vary in their cultural and physiological characters may possibly be correlated with the author's experience here with regard to the pathogenicity of the Brown Rot organism, i.e., its inability to attack tobacco, thus suggesting that the organism attacking tobacco in New South Wales is a different strain. As pointed out in the introduction to this paper, Tryon (6) in 1894 described a bacterial disease of potatoes in Queensland. Without adequate proof of its pathogenicity, and without describing it completely, he named the organism *Bacterium vasculareum solani*. After E. Smith's paper in 1896 (4), establishing a bacterial organism as the cause of Brown Rot of potato in the United States, and naming it, after adequate description, *B. solanacearum*, Tryon (7) in 1899 published a paper claiming priority for his name. Smith (5) replied that he could not use the name as (a) Tryon had not published an exact description of the disease, (b) he had not published an exact description of the organism. Tryon's description of the disease tallies in many ways with the Sore Eye disease in Victoria, and there seems little doubt that he was dealing with the same disease. Some parts of the description would seem to suggest, however, that he was examining Black Leg diseased plants as well. The author feels supported in his view that such is the case since in Victorian potato growing areas where Sore Eye disease is found, Black Leg disease also occurs.

The conclusion arrived at as a result of this investigation is that a strain of *Bacterium solanacearum* E.F.S. causes Brown Rot or Sore Eye of potatoes in Victoria, and that it is highly probable that the same, or a slightly different strain, causes Brown Rot of potatoes in other States of the Commonwealth.



Brown Rot Disease.

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Explanation of Plate.

- Fig. 1.—Naturally infected potato plant showing the wilted appearance characteristic of the disease.
- Fig. 2.—Infected tubers showing particles of earth adhering round the eyes. This is due to the sticky nature of the bacterial exudate from the eyes.
- Fig. 3.—Tuber cut in halves to show the bacterial ooze from the vascular system.
- Fig. 4.—Microphotograph of a transverse section of an infected stem showing the bacteria clogging the wood vessels.
- Fig. 5.—Artificially infected tomato plant. Very marked recurving of the petioles occurs before wilting becomes apparent.